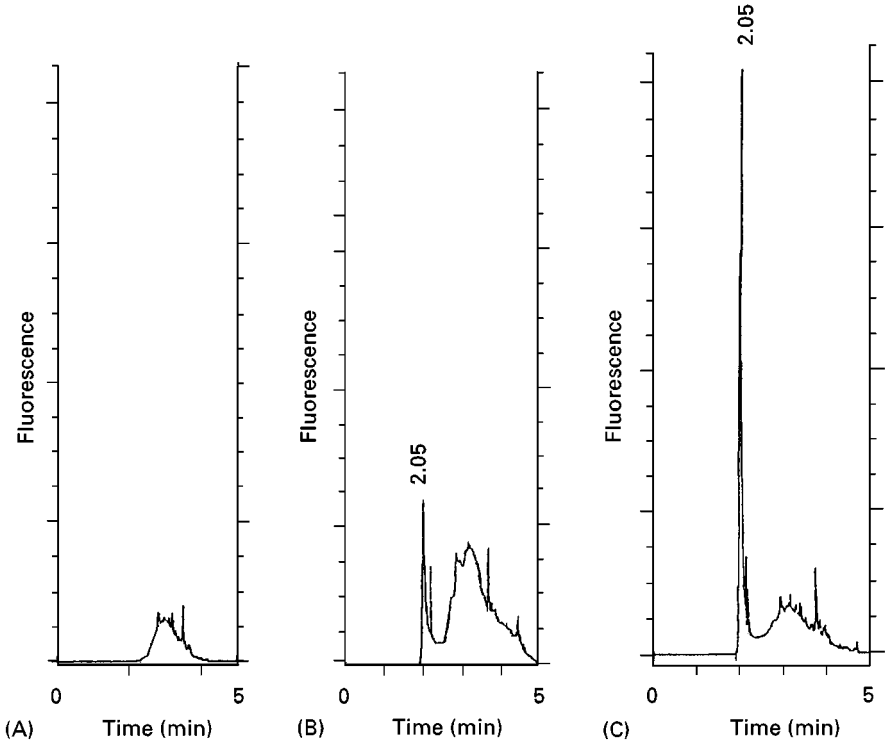

Figure 7 Quantification of human immunoglobulin G (h-IgG) using FITC-labelled Protein G. A, no IgG; B, 250 μg mL^{-1}; C, 1 mg mL^{-1}. (Adapted with permission from Reif O-W, Lausch R, Scheper Th and Freitag R (1994) *Analytical Chemistry* 66: 4027–4033.)

Further Reading

DropBooks

Beale SC (1998) Capillary electrophoresis. *Analytical Chemistry* 70: 279. (This journal prints every two years a review on capillary electrophoresis. The article is written by an expert in the field and covers more or less all developments in CE including CGE of the preceding years.)

Grossmann PD and Colburn JC (eds) (1996) *Capillary Electrophoresis: Theory and Practice*. San Diego: Academic Press.

Grossmann PD and Soane DS (1991) Capillary electrophoresis of DNA in entangled polymer solutions. *Journal of Chromatography* 559: 257. This paper treats the two models for separation in CGE (Ogston and reptation) in more detail.

Jörgenson JW and Lukacs KD (1981) High resolution separation based on electrophoresis and electro-osmosis. *Analytical Chemistry* 53: 1298.

Kuhn R and Hoffstetter-Kuhn S (1993) *Capillary Electrophoresis: Principles and Practice*. New York: Springer Verlag.

Landers JP (ed.) (1994) *Handbook of Capillary Electrophoresis*. Boca Raton: CRC.

Li SFY (1992) *Capillary Electrophoresis*. Amsterdam: Elsevier.

Lunte SM and Radzik DM (1996) *Pharmaceutical and Biomedical Applications of Capillary Electrophoresis*. Oxford: Pergamon Press.

Schwartz H and Guttman A (1995) *Separation of DNA by Capillary Electrophoresis*. Fullerton, CA: Beckmann Primer 607397.

Capillary Isoelectric Focusing

P. G. Righetti, University of Verona, Faculty of Sciences, Verona, Italy
C. Gelfi, ITBA, CNR, Segrate, Milan, Italy

Copyright © 2000 Academic Press

Isoelectric focusing (IEF) possibly represents the electrokinetic method with the highest resolving power. In IEF, amphoteric compounds are sorted in order of their isoelectric points (p*I*) in a steady-state pH gradient. Good resolution is favoured by both a low diffusion coefficient and a high mobility slope at the p*I*,

conditions which are well satisfied by most proteins. A high field strength and a shallow pH gradient further enhance resolution. There are two basic variants of IEF: (1) in soluble, amphoteric buffers, called carrier ampholytes (CA) and (2) in insolubilized, non-amphoteric buffers (the latter technique is known as immobilized pH gradients, IPG). In this article we will deal with the former, i.e. CA-driven IEF, since IPGs have not as yet been implemented in capillaries. In fact, in IPGs, the buffers (acrylamido weak acids and bases) have to be grafted onto a support which at present is only a polyacrylamide gel. In addition, the gradient is created 'artificially', outside the electric field (whereas in CA-IEF the pH gradient has to be generated and maintained by the electric field itself), and thus gel casting requires the use of a two-vessel gradient mixer, with the simultaneous pouring of a density and a pH gradient in a flat-gel slab format. In normal use IPGs additionally require that the gel cassette is opened and that the polyacrylamide slab, with the grafted pH gradient, is exhaustively washed, so as to eliminate salts, ungrafted buffers and catalysts. This preparation sequence means that preparing an IPG in a capillary format is not a straightforward process.

The theory of IEF and IPGs, as well as the chemistry of the buffers adopted, has been covered elsewhere. Here we will focus on the techniques used in capillary IEF (CIEF), namely: one- and two-step focusing methods, pH gradient determination, sample preconcentration systems and some application examples.

General Considerations

CIEF combines the high resolving power of conventional IEF with the advantages of automation and speed. The separation of charged analytes takes place in a pH gradient created in a capillary by carrier ampholytes under the influence of an electric field. The concentrating effect that occurs during the focusing step, enables components present in small quantities to be detected. A major difference between IEF and CIEF is the detection method: in CIEF detection is performed in most cases with an ultraviolet (UV) or photodiode array detector placed near one end of the capillary. In order to visualize the stationary zones formed in the capillary, its content must therefore be mobilized in an additional step so that they pass in front of the detector window. To date, most of the reagents used in CIEF (carrier ampholytes, solubilizers and so on) have been adopted from traditional IEF. A number of reviews have already appeared on the topic of CIEF (see the Further Reading section).

A principal difference between IEF in a gel and in a capillary is that, in the latter, mobilization of the focused proteins past the detector has to be carried out if an on-line imaging detection system is not used. Three techniques are mainly used: chemical and hydrodynamic flow mobilization (in coated capillaries) and mobilization utilizing the electrosmotic flow (in uncoated or partially coated capillaries). These techniques are discussed further below.

Focusing in Internally Coated Capillaries

At any pH value above pH 2 the fused silica surface will progressively acquire negative charges due to ionization of weakly acidic silanol groups which are fundamental constituents of any vitreous material. Accordingly, close to the capillary wall (in the diffuse part of the double layer) there will be more positive than negative ions (electroneutrality will thus not prevail in the double layer). In an electric field, the hydrated, positively charged surface layer will move toward the negative pole, thus producing an electrosmotic flow (EOF), which can be observed as a bulk fluid movement. Such an EOF pump is not, in itself, deleterious to the analyte zone, since it has a flat profile (except in the few nanometre thickness of the double-layer); however, in the case of proteins, strong adsorption may ensue, due to multipoint attachment of positively charged species to the negative charges of the wall. In addition, particles migrating in directions opposite to the bulk liquid flow might never reach the detector. The electrosmotic mobility (μ_{eo}) is inversely proportional to the viscosity (in the double layer). Thus, by coating the inner surface of the capillary with a hydrophilic, non-ionic polymer, there will be two beneficial effects: the charges will be masked and, in general, suppressed and, additionally, the viscosity in the double layer will be so high as to virtually eliminate EOF. A typical coating consists in first reacting the wall with a bifunctional agent (γ-methacryloxypropyltrimethoxysilane) and then covalently affixing a monolayer of linear polyacrylamide to the dangling double bonds. Many other coating procedures have been described and have been reviewed by Chiari *et al.* (1996).

Focusing Step

The coated capillary is filled entirely with sample solution mixed into at least 1% carrier ampholytes (the sample should be desalted so as to avoid pH gradient drift). One end of the tube is then pressed into a 1% agarose gel, prepared either in 20 mM NaOH or in 20 mM phosphoric acid (representing

the cathodic and anodic solutions, respectively). The gel plug thus inserted into the tube end prevents zone-deformation by hydrodynamic flow in the tube during the subsequent focusing step. A constant voltage of 4000–6000 V is applied. When the steady-state has been attained, which occurs when the current has dropped to about 10–25% of the starting value, the voltage is switched off and elution started immediately as described below.

Elution and Detection Step

In such a system, due to suppression of EOF, the focused stack of carrier ampholytes and proteins is arrested; thus ways have to be found to transport the stack past the detector. A number of procedures can be adopted: (1) apply a mechanical pump to the capillary and generate a hydrodynamic flow (at the end of the IEF process); (2) replace the base at the cathode with acid or the acid at the anode with base and (3) salt mobilization. This latter technique has attained wide popularity. When a salt (e.g. NaCl) is added at the anolyte, mobilization will be towards the anode; conversely, if added to the catholyte, the train of bands will elute at the cathode. **Figure 1** shows a representation of this process: the upper part depicts the steady-state, characterized by a stationary pattern of focused proteins in an arrested pH gradient. In this stage, current is carried mostly by protons and hydroxyl ions moving from the anodic and cathodic compartments, respectively (and also by the to and fro movement of the CA buffers about the p*I* position!). On addition of the salt (typically 20–80 mM) at the cathodic reservoir, the stack of proteins and CA buffers is mobilized towards the cathodic side (past the detector). The time required for mobilization is about 15 min at 360 V cm^{-1}. During mobilization the current which had reached a minimum at the end of the focusing stage (typically 1 μA) rises again to as high as 50 μA. It is during mobilization that the train of zones, titrated away from the p*I* by the cations or anions (other than protons or hydroxyl ions) entering the tube from one of the electrode reservoirs, transits in front of the detector and is registered as a series of bands. Ideally, proteins and peptides should best be monitored at 210 (or even 190) nm where the absorbance of the amido bond is 20–50 times higher than at 280 nm. However, at the low wavelengths the CA buffers also produce a UV signal (rather similar for Ampholine and Biolyte, quite different in the case of Pharmalytes) which could be mistaken as sample zones. Thus, in an IEF experiment, it is best to read the sample at 280 nm.

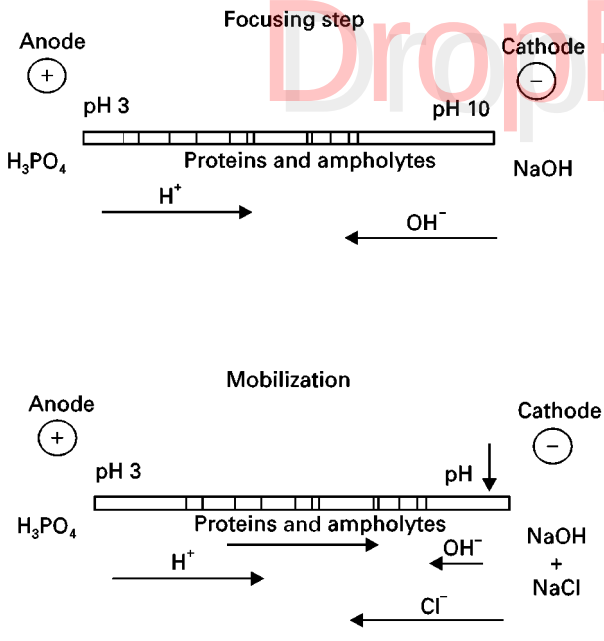

Figure 1 Focusing in internally coated capillaries. The focusing (upper) and mobilization (lower) steps. In the upper drawing the steady-state is shown as an arrested stack of proteins and CA buffers. Only protons and hydroxyl ions move from the respective electrodes carrying most of the current. In the lower drawing, addition of NaCl to the cathode is shown to mobilize the stack of proteins and CA buffers towards and past the detector port (represented as a large vertical arrow close to the cathode. (Reproduced by permission of Bio Rad, Hercules, CA, USA.)

DropBooks

Elution by Vacuum or Pressure Under Voltage

An alternative elution method consists in applying a vacuum of 5 mmHg while still under high voltage. The vacuum causes the focused proteins to flow past the detector, while the voltage maintains the pH gradient and zone sharpness even in the presence of distorting effects due to laminar flow. First, the entire tube is filled with NaOH (catholyte). Then, by hydrodynamic flow, approximately two-thirds of the capillary length is filled with carrier ampholytes. This is followed by a short sample plug, which is subsequently insulated from strong anolyte (which might, by contact, denature some proteins). At this point liquid pumping is stopped and the focusing process takes place between the phosphoric acid as anolyte and the NaOH as catholyte. Mobilization is again accomplished by a vacuum-driven hydrodynamic flow under voltage. The detection limit for proteins at 280 nm is as low as 1.3 ng, while the signal linearity is in the range of 1.3–10.7 ng (an eight-fold concentration range). Interestingly, this sensitivity is of the same order of magnitude as that reported for silver staining of sodium dodecyl sulfate-denatured protein zones and two to three orders of magnitude higher than conventional Coomassie Brilliant Blue staining.

p*I* Measurements

In its simplest approach unknown p*I* values can be assessed by plotting the p*I* values of a set of markers, co-focused with the proteins under investigation, versus their relative mobility on elution. This plot is linear and thus a high precision (approximately $\pm$ 0.1 pH unit) is obtained (see **Figure 2**). p*I* values as low as 2.9 and 2.75 can be determined. In another approach monitoring the current in the mobilization step can be adopted for p*I* assessments. If the peaks of the mobilized stack of proteins are monitored simultaneously with the rising current due to the passage of the salt wave in the capillary one can correlate a given p*I* value (which should already be known from the literature) with a given current associated with the transit of a peak at the detector port. The system can thus be standardized and used for constructing a calibration graph to be adopted in further work, without resorting to 'internal standards'. One such graph correlating current with p*I* values is shown in **Figure 3**: this appears to be a precise method, since the error is only about 0.03 pH units. The use of low M_r substituted aromatic aminophenols, which fulfil all the requirements for pI standards for CIEF and assure a 0.06% pI reproducibility, have also been proposed. In another method dansylated peptides have been synthesized for use as pI markers for evaluating pH gradient formation.

On Isoelectric Precipitation

Proteins have nett negative and nett positive charges at pH values above and below their p*I* values. This decreases the risk of aggregation, which ultimately may lead to precipitation. However, at, or in the proximity, of their p*I* value proteins exhibit a minimum of total charge and thus a solvation minimum. This increases the risk of aggregation, and is further enhanced by the extremely low ionic strength conditions prevailing in IEF. When protein molecules precipitate they probably aggregate by hydrophobic interactions. It seems logical therefore to try to suppress precipitation by supplementing the CA buffers with agents known to decrease hydrophobic interactions, such as ethylene glycol (10–40% v/v) or detergents (1–4% w/v). The detergents should be either non-ionic or zwitterionic, so as to be compatible with the focusing process; in addition, they should preferably be transparent at 280 nm so as to minimize interference with protein detection. There are some simple ways to reduce protein interaction and precipitation: one is to use dilute protein solutions (aggregation is proportional to protein concentration); the other is to increase the CA buffer concentration up to 4%, since this leads to an increase in total ionic strength. Conti *et al.* (1997) have investigated the use of a large number of solubilizers and proposed mixtures of mild agents capable of fully preserving the three-dimensional structure and full activity of biomolecules. Among the solubilizing agents are non-detergent sulfobetaines, taurine, Good's buffers such as bicine and CAPS, polyols, such as sucrose, sorbose, sorbitol and mixtures thereof.

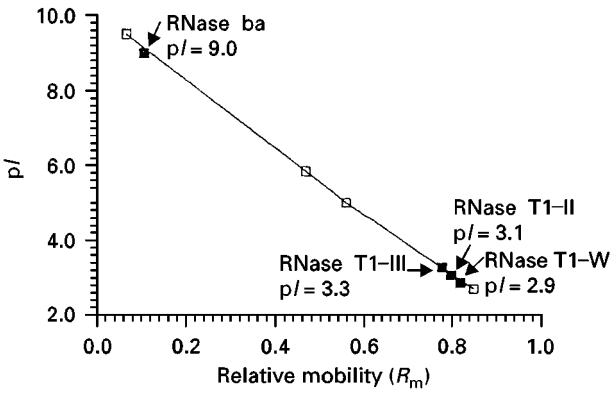

Figure 2 Calibration graph for p*I* determination using a set of marker proteins. The markers (open squares) are ribonuclease A (p*I* 9.45); carbonic anhydrase (p*I* 5.90); β-lactoglobulin (p*I* 5.1) and unsulfated cholecystikinin flanking peptide (p*I* 2.75). The four solid squares represent four unknown proteins whose p*I*s have been determined by linear interpolation in the calibration graph. (Reproduced from Chen SM and Wiktorowicz JE (1992) *Analytical Biochemistry* 20: 84–98 by permission.)

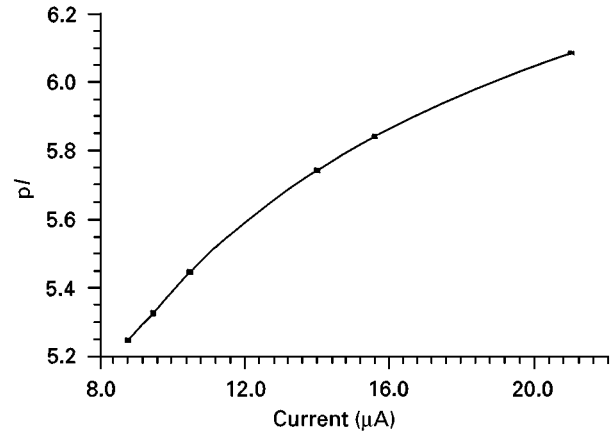

Figure 3 Calibration graphs for p*I* determination using the current during the mobilization step as a parameter in capillary IEF. The six experimental points represent six forms of transferrin, containing different amounts of sialic acid and of iron. (Reproduced from Kilar F (1991) *Journal of Chromatography* 545: 403–406, by permission.)

The Detection System

As discussed above, the standard absorption wavelength for detection in IEF is 280 nm, the typical

absorption maximum of proteins. As an alternative method a universal concentration gradient imaging system not requiring a mobilization step can be used. This system is based on the Schlieren shadowgraph methods and uses a He–Ne laser to probe the capillary content and a charge-couple device (CCD) as a detector. The capillary has to be square, not covered by polymide and rather short (4 cm). Focusing is completed in 2 min, resolution is of the order of 0.02 pH units and the mass detection limit appears to be of the order of the picomole level. As an alternative, a whole column UV absorbance detection consisting in transporting the entire column past the detector (the capillary, of course, has to be UV transparent) can be used. In this set-up the optical configuration consists of a 5 mW argon ion laser (with either a 496.5 or a 514.5 nm lasing line) probing the entire length of a 4 cm long, square glass capillary and using as a detector a 1204-pixel CCD camera. Focusing and imaging are usually completed in 2–4 min. The problem is that only coloured proteins (those that absorb in this wavelength region) can be efficiently detected (e.g. haemoglobin, myoglobin and cytochrome-c). Finally, a mass spectrometer has been proposed as a detector after CIEF: it should be noted that the technique becomes two-dimensional, since proteins are then mapped by both charge and mass.

DropBooks

Focusing in Dynamically Coated Capillaries

Three groups have reported approaches on the possibility of focusing in dynamically coated capillaries. The techniques will be reviewed below.

Dynamic Coating with Methylcellulose

Rather than completely eliminating EOF one might try to reduce it to such an extent as to allow attainment of steady-state conditions; from there on, the bulk flow would keep the 'arrested' stack moving past the detection window. This approach would then obviate the need for performing salt, vacuum or hydrodynamic mobilization; focusing and elution being accomplished in one step. A simple way for modulating EOF is to add viscous polymer solutions. The capillary can be conditioned with 0.1% methyl cellulose, mixed with sample and CA buffers and used to fill the entire column. It has been found that by increasing the anolyte concentration to 25 mM phosphoric acid also allows the detection of acidic proteins. Finally, it has been assessed how deleterious different amounts of salt (NaCl) present in the sample would be to the focusing process. As little as 10 mM of NaCl in the sample suffices to entirely destroy the separation so salt in the analyte must be kept below this level.

Dynamic Coating with Hydroxypropyl Methylcellulose

In another system, the dynamic agent used for partial coating is hydroxypropyl methylcellulose (HPMC). In this approach, some interesting variants have been adopted. A new capillary is first rinsed for 20 min with 1 M NaOH and then for 10 min with 0.1 M NaOH containing 0.3% HPMC. It is during this final washing that conditioning of the capillary and partial coating with HPMC occurs. This etching procedure (with 1 M NaOH), followed by a short renewal of the dynamic coating (0.3% HPMC in 0.1 M NaOH) is shown to provide data of the highest reproducibility. The sample proteins are dissolved in 2.5% Ampholine solution, without any addition of HPMC. The anolyte is the standard 10 mM phosphoric acid solution, whereas the catholyte consists of 20 mM NaOH in the presence of 0.1% HPMC. The sample is introduced as a plug, occupying only 10–50% of the capillary length at the anodic side, the remaining being filled with catholyte. Since the entire stack of proteins will eventually be displaced towards the cathode by the EOF, this initial sample plug distribution allows more time to reach a good focusing pattern prior to sample passage in front of the detector.

Dynamic Coating with Adsorbed Surfactants or Polymers

In another variant, reduction of EOF via derivatization of capillaries with a hydrophobic coating (octadecylsilane) followed by adsorption of either a surfactant (Brij 35, PF-108) or a hydrophilic polymer (e.g. polyvinyl alcohol, polyvinyl pyrrolidone, methylcellulose) has been proposed. The procedure is as follows: the capillary is first treated with 1 M NaOH for 30 min, followed by several washings with deionized water and methanol (30 min each). The residual methanol is evaporated in an oven at 90°C for 2 h, while flushing the capillary with a stream of nitrogen at 400 kPa. While still in the oven at 90°C, a solution of octadecyltrichlorosilane in 50% toluene is flushed through the capillary for 6 h. After silylation, the capillary is rinsed for 20 min with methanol and then with water for 30 min. Surfactant solutions (in general 0.4%) are pumped continuously through the capillary for an additional 6 h in order to complete the coating process. Coating via adsorption of detergent (or methylcellulose 4000) is shown to reduce the EOF of the native, untreated capillary, to approximately 3–5% of the original value but the

residual EOF still allows adequate flow to obviate the need for a separate mobilization step. Based on the resolution of haemoglobin variants proteins that varied 0.03 pH units in isoelectric point were resolvable.

Sample Preconcentration Systems

Typical preconcentration steps commonly used in biochemical analysis (especially for macromolecules) such as lyophilization, ultrafiltration, partition between two polymer aqueous phases, osmotic removal of water and chromatographic adsorption-desorption, will entail large losses when the sample volume is 1–10 μL or less, as is customary in capillary zone electrophoresis (CZE). A number of electrophoretic methods for concentrating biopolymers (especially peptides and proteins), while partially depleting them of strong electrolytes (often a problem in all IEF procedures), have been described. In practice the whole electrophoresis tube is filled with the sample solution to be concentrated and then the sample is allowed to migrate against the end of the tube where a gradient of conductivity or viscosity exists and is arranged in such a way as to continuously slow down sample electrophoretic migration. The sample will finally collect in a narrow zone of the tube (typically 0.2–0.5 mm in width). A 400–1000-fold concentration is obtained when a 200 mm long tube is filled completely with the sample and still more if an electrode vessel is also loaded with sample. As an alternative, an on-line isotachophoretic (ITP) concentration process of very large injection volumes prior to CZE analysis can be adopted. Sample volumes up to 25 μL can be concentrated by this system. As concentrating large volumes would take a relatively long time, depending on the migration path length, a system of coupling a narrower bore to a larger bore capillary is generally utilized in order to speed up the process. Finally, after the ITP concentration step the sample can be analysed by CZE via a T-junction connected to another electrolyte reservoir (i.e. one has to resort to a three-pole column).

Some Application of Examples

A vast body of applications already exists; a number of them can be found in the reviews listed in the Further Reading section. We will consider some selected examples here. By using umbilical cord blood which contains only three major haemoglobin (Hb) components (Hb F, Hb A and Hb F acetylated, F_{ac}) it is possible to perform thalassaemia screening provided a good separation is obtained between Hb A and Hb F_{ac}, which have minute differences in p*I* values. In order to improve the separation the pH 6–8 Ampholine is admixed with an equimolar mixture of 'separators', namely 0.2 M β-alanine and 0.2 M 6-aminocaproic acid, which flatten the pH gradient in the focusing region of the three major components. **Figure 4** shows this separation obtained by CIEF. The method is simple, can unambiguously detect any

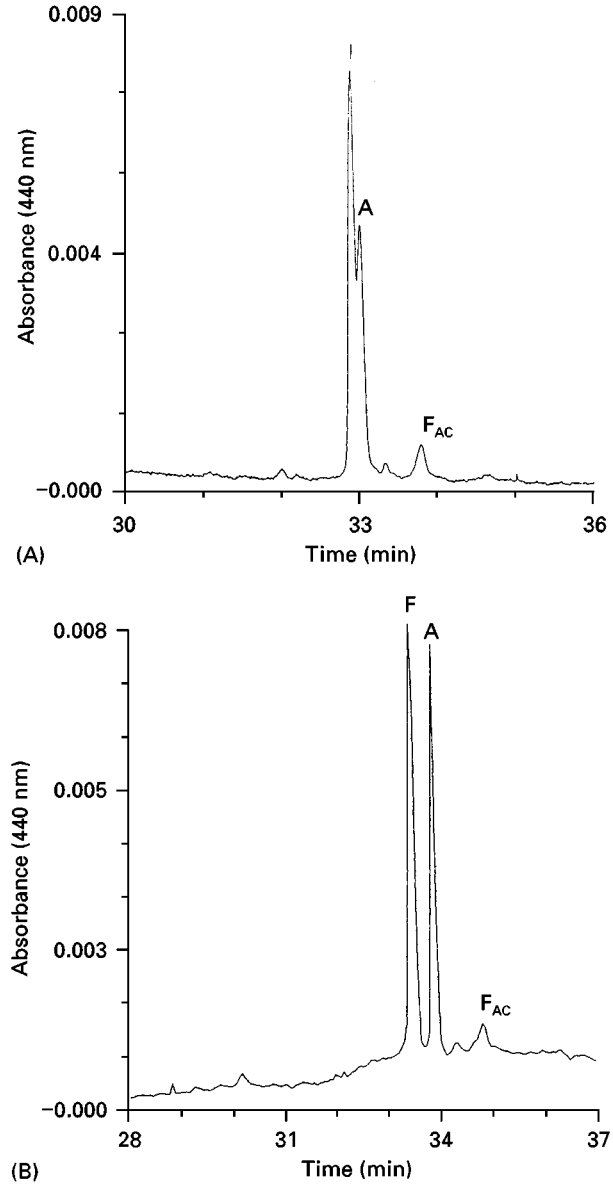

Figure 4 Separation of Hb F, A and F_{ac} by capillary IEF. Background electrolyte: 5% Ampholine, pH 6–8, added with 0.5% TEMED (panel A) and additionally with 3% short-chain polyacrylamide and 50 mM β-Ala (panel B). Anolyte: 20 mM H_3PO_4; catholyte: 40 mM NaOH. Sample loading: by pressure, for 60 s. Focusing run: 20 kV constant at 7 μA (initial) to 1 μA (final current), 20°C. Capillary: coated with poly(AAEE), 25 μm internal diameter, 23.6/19.1 total/effective length. Mobilization conditions: with 200 mM NaCl added to anolyte, 22 kV. Detection at 415 nm. (Reproduced from Conti M, Gelfi C and Righetti PG (1995) *Electrophoresis* 16: 1485–1491, by permission.)

thalassaemic condition and can be easily performed on a routine basis in a neonatal unit. Using the same principle Conti *et al*. have also attempted CIEF separation of Hb A from Hb A_{1c} (the glycated form of Hb A), the latter component being of diagnostic value for the long-term control of diabetic patients (glucose

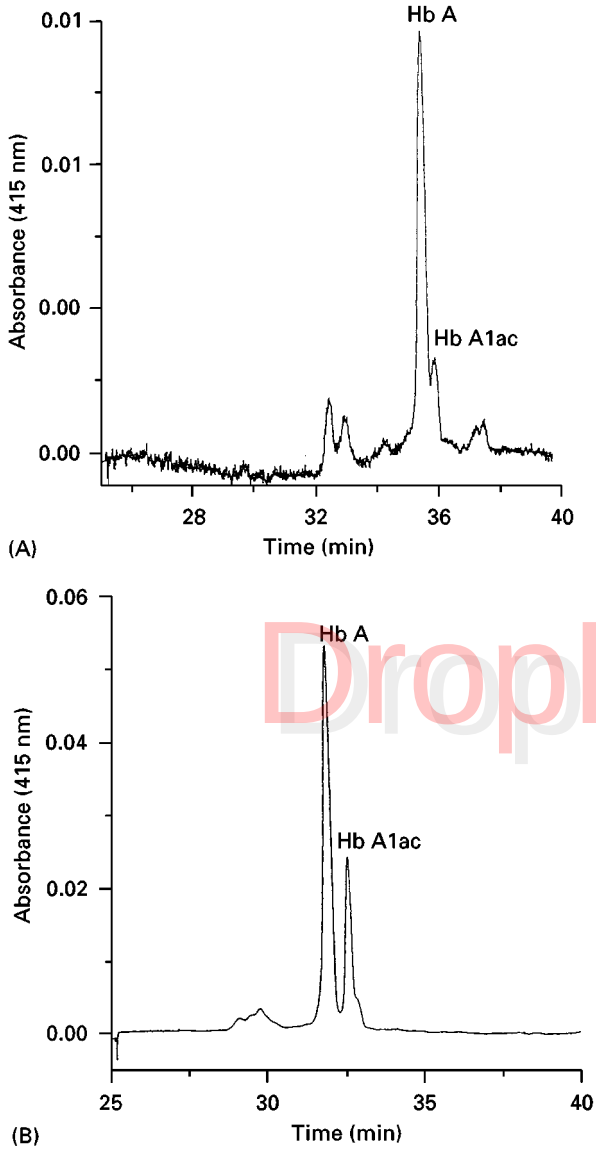

Figure 5 Separation of Hb A from A_{1c} by capillary IEF in the absence (A) and in presence (B) of 3% short-chain polyacrylamide and an equimolar mixture of 'separators', 0.33 M β-Ala and 0.33 M 6-amino caproic acid. Background electrolyte: 5% Ampholine, pH 6–8, added with 0.5% TEMED. Anolyte: 20 mM H_3PO_4; catholyte: 40 mM NaOH. Sample loading: by pressure, for 60 s. Focusing run: 20 kV constant at 7 μA (initial) to 1 μA (final current), 20°C. Capillary: coated, 25 μm internal diameter, 23.6/19.1 total/effective length. Mobilization conditions: with 200 mM NaCl added to anolyte, 22 kV. Detection at 415 nm. (Reproduced from Conti M, Gelfi C, Bianchi-Bosisio A and Righetti PG (1996) *Electrophoresis* 17: 1590–1596, by permission.)

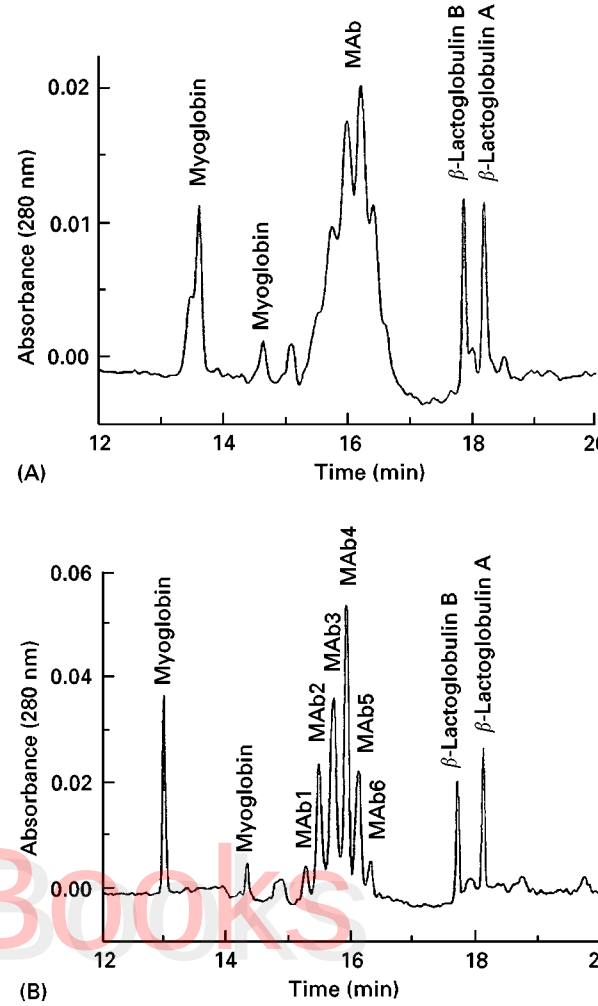

Figure 6 CIEF of a mouse monoclonal antibody using pressure mobilization. Focusing for 2 min at 10 kV, followed by mobilization at low pressure (0.5 psi) at (A) 10 kV and (B) 20 kV. Concentration of the marker proteins: 50 ng μL^{-1}; concentration of desalted antibody: 0.5 μg μL^{-1}. Ampholyte solution: 4% Pharmalyte, pH 3–10, 1% TEMED in 0.8% methyl cellulose. Anolyte: 10 mM H_3PO_4; catholyte: 20 mM NaOH. (Reproduced from Schwer C (1995) *Electrophoresis* 16: 2121–2126, by permission.)

DropBooks

binds irreversibly to Hb molecules; the percentage of Hb A_{1c} varies with the blood glucose concentration to which red blood cells have been exposed during their circulating lifetime, see **Figure 5**). A good separation of monoclonal antibodies is shown in **Figure 6**: as mobilization was obtained by pressure under voltage it shows the importance of working under high voltage during this step.

Further Reading

Chiari M, Nesi M and Righetti PG (1995) Surface modification of silica walls: a review of different methodologies. In Righetti PG (ed.) *Capillary Electrophoresis in Analytical Biotechnology*. Boca Raton: CRC Press. pp. 1–36.

Conti M, Galassi M, Bossi A and Righetti PG (1997) Capillary isoelectric focusing: the problem of protein solubility. *Journal of Chromatography A* 757: 237–245.

Liu X, Sosic Z and Krull IS (1996) Capillary isoelectric focusing as a tool in the examination of antibodies, peptides and proteins of pharmaceutical interest. *Journal of Chromatography A* 735: 165–190.

Pritchett TJ (1996) Capillary isoelectric focusing of proteins. *Electrophoresis* 17: 1195–1201.

Righetti PG (1983) *Isoelectric Focusing*: *Theory*, *Methodology and Applications*, Amsterdam: Elsevier.

Righetti PG (1990) *Immobilized pH Gradients: Theory and Methodology*. Amsterdam: Elsevier.

Righetti PG and Bossi A (1998) Isoelectric focusing of proteins and peptides in gel slabs and in capillaries. *Analytica Chimica Acta* 372: 1–19.

Righetti PG, Gelfi C and Conti M (1997) Current trends in capillary isoelectric focusing of proteins. *Journal of Chromatography B* 699: 91–104.

Righetti PG, Bossi A and Gelfi C (1997) Capillary isoelectric focusing and isoelectric buffers: an evolving scenario. *Journal of Capillary Electrophoresis* 4: 47–59.

Rodriguez-Diaz R, Wehr T and Zhu M (1997) Capillary isoelectric focusing. *Electrophoresis* 18: 2134–2144.

Steinmann L, Mosher RA and Thormann W (1996) Characterization and impact of the temporal behaviour of the electroosmotic flow in capillary isoelectric focusing with electroosmotic zone displacement. *Journal of Chromatography A* 756: 219–232.

Strege MA and Lagu L (1997) Capillary zone electrophoresis and isoelectric focusing of biotechnology-derived proteins. *Electrophoresis* 18: 2343–2352.

Taverna M, Tran NT, Merry T, Horvath E and Ferrier D (1998) Electrophoretic methods for process monitoring and the quality assessment of recombinant glycoproteins *Electrophoresis* 19: 2527–2594.

Capillary Isotachophoresis

J. Sádecká and J. Polonský, Faculty of Chemical Technology, Bratislava, Slovak Republic

Copyright © 2000 Academic Press

Introduction

Isotachophoresis (ITP) is one of the fundamental electrophoretic separation techniques, where charged constituents are separated in an electric field due to their differences in their electrophoretic mobilities.

The moving boundary electrophoretic experiments and theoretical developments were the forerunners of isotachophoresis. Even by 1923, Kendall and Crittenden had described separation of some metals and acids by – as they called it – the 'ion migration method', which was in fact isotachophoresis. They concluded that ion concentrations were in accordance with the Kohlrausch regulation function. In 1942, Martin did his first experiments on what he called 'displacement electrophoresis' as an analogue of displacement chromatography. In 1963, Everaerts and Martin started their work on isotachophoresis. Up to 1970 several names had been used for what Kendall had called the ion migration method: these included the 'moving boundary method', 'displacement electrophoresis', 'steady-state stacking', and 'ionophoresis'. In 1970, Haglund introduced a name, based on the characteristic feature of the electrophoretic technique, namely the equal velocity of the sample zones in the steady state: isotachophoresis (ITP). The basic theory and early development in the field of isotachophoresis was described in 1976 by Everaerts in his fundamental book. An outline of the development of isotachophoresis is given in **Table 1**. Some advances in isotachophoresis are described in detail below.

ITP in Closed Systems

Up to 1990, ITP was carried out in commercial apparatus in 200–500-μm i.d. narrow-bore plastic capillaries and with closed systems, i.e. no electroosmotic flow (EOF) occurred.

Basic Theory

Under the influence of an applied electric field, E, ionic species will move towards the electrode with a migration velocity, v, of:

$$v = m \times E \qquad [1]$$

where m is the effective mobility of an ionic species. The effective mobility depends on various factors, for example, the ionic radius, shape and charge of the ion, degree of dissociation, pH, dielectric constant and viscosity of the solvent, and temperature.

Typically, ITP is performed with a constant current and it is not possible to separate cations and anions in the same run (unidirectional isotachophoresis).

It is characteristic of ITP that the sample to be separated is injected between two different electrolyte solutions. The first solution (the leading electrolyte) contains an ion (the leading ion) with the same charge